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<p>(54) Title: METHOD OF ACTIVATING PHOTSENSITIVE AGENTS</p> <p>(57) Abstract</p> <p>A method of administering photodynamic therapy begins with administering to an animal an effective amount of a photosensitizing agent which is less than about one half of the usual clinical dose for the photosensitizing agent. Then, following a post injection interval which is less about one quarter of the usual post injection interval, an effective dose of light which is less than about one half of the usual clinical dose of light used in conjunction with the photosensitizing agent is administered to the animal.</p>		

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METHOD OF ACTIVATING
PHOTOSENSITIVE AGENTS

Field of the Invention

This invention relates generally to the
10 field of medicine and pharmacotherapeutics with
photosensitizing agents. Specifically, the invention
is a method of destroying target tissue which entails
administration of a photosensitizing agent and
application of radiation to selectively impair or
15 destroy the target tissue.

Background of the Invention

Photodynamic therapy (PDT) involves the
administration of a photosensitizing compound and
20 subsequent irradiation with light of tissue in which
the photosensitizing compound has concentrated.
Target tissue containing a sufficiently high
concentration of the photosensitizing compound
selectively absorbs the light which induces impairment
25 or destruction of the immediately surrounding cells.
U.S. Patent 5,095,030 issued to Levy on 10 March 1992
describes procedures for administering
photosensitizing compounds to animals which are
subsequently irradiated using external light sources.
30 For example, Example 5 of this patent describes
subcutaneous injection of mice with P815 tumor cells
which grow into a palpable tumor. Photosensitizing
compounds are then injected. Then the animals are
maintained in the dark for two hours. Next, their
35 tumors were exposed to a strong light. The survival
rates of the treated animals were significantly

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5 improved over untreated controls. Similarly, Example
8 of that patent describes use of a rhabdomyosarcoma
system in mice with a similar protocol. However, in
this case light exposure commenced 24 hours post
injection. In addition, biodistribution of tritiated
10 BPD-MA and BPD-MB was determined post injection at
times ranging from 3-168 hours. Tumor-skin ratios
were favorable three hours after IV administration.
Biodegradability was determined with tritiated BPD-MA
injected IV into PB15 tumor-bearing mice. Mice were
15 sacrificed at three or 24 hours after BPD-MA
injection; and tumors, livers and kidneys were
evaluated. After 3 hours 100% of BPD-MA in the tumor
was active, but only 39% was active at 24 hours. Both
livers and kidneys degraded BPD-MA more quickly than
did the tumors.

20 Kostron et al. (J. Neuro-Oncology (1988)
6:185-91) injected hematoporphyrin derivative directly
into subcutaneous rat gliosarcomas and irradiated 48
hours post injection. Kostron reported that direct
injection appeared to be safer than parenteral
25 injection. Kostron also mentioned previous studies
indicating that there should be a post injection delay
of at least two days and preferably three to four days
before light was applied because that would permit the
hematoporphyrin derivative to concentrate in tumor
30 cells.

BPD also has demonstrated a higher affinity
for tumor tissue, including leukemic cells, than for
normal non-malignant cells. Jamieson et al., Leukemia
35 Res. 14:209-19, 1990. Photosensitizers also are
useful in the detection and treatment of
atherosclerotic plaque as described in U.S. Patent

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5 Nos. 4,521,762 and 4,577,636. The treatment of viral
diseases is disclosed in U.S. Patent Nos. 4,878,891,
issued 7 November 1989 to Judy et al.; 4,925,736,
issued 15 May 1990 to Shikowitz; and 4,935,498.
Psoriasis treatment is disclosed in U.S. Patent No.
4,753,958, issued 28 June 1988 to Weinstein et al.
10 Arthritis treatment is disclosed in U.S. Patent No.
5,028,994, issued 2 July 1991 to Carson. Portwine
stain treatment is disclosed in Canadian patent
publication CA 2,012,175.

15 U.S. Patent No. 5,095,030, issued 10 March
1992, which is incorporated herein in its entirety by
reference, discloses and claims various wavelength-
specific cytotoxic agents which are generically
described as "green porphyrins." These compounds are
porphyrin derivatives which are modified by a Diels-
20 Alder reaction effectively to shift the wavelength of
absorption to a longer wavelength. This results in
some favorable properties as compared to, for example,
hematoporphyrin derivative when these compounds are
employed in photodynamic therapy generally. As
25 described in this patent, these cytotoxic agents, when
administered systemically, "home" to unwanted cells,
in particular to tumor cells or viruses. Subsequent
irradiation with light absorbed by these compounds is
cytotoxic.

30 Pending Application Serial No. 07/832,542,
filed February 5, 1992, which is incorporated herein
in its entirety by reference, discloses the
preparation of liposomes of porphyrin
photosensitizers.

35 Pending Application Serial No. 07/948,113,
which is incorporated herein in its entirety by

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5 reference, discloses the injection of BPD into mice to
treat blood-borne target cells. This application also
discloses pharmacokinetic data at post injection
intervals between 15 minutes and two hours. All mice
given doses of 6.32 $\mu\text{g/ml}$ and illuminated starting at
15 minutes post injection died. However, other mice
10 injected with lower BPD doses or longer post injection
times (e.g., one hour) remained healthy.

Adverse effects following the administration
of PROTOFRIN[®] porfimer sodium have been documented by
Dougherty et al. Lasers in Surg. Med. (1990) 10:485-
15 88; and Harty et al. J. Urology (1989) 141:1341-46.
In a series of 180 patients treated with porfimer
sodium, Dougherty reported that patients received 0.5
to 2.0 mg/kg to treat a variety of cancers but did not
mention light dose or post injection interval before
20 light treatment. However, the recommended post
injection interval for this drug is 24-48 hours.
Dougherty cautions that "all patients are
photosensitive following injection of Photofrin."
Treated patients were polled in person and through
25 questionnaires about photosensitivity reactions. The
in-person reports of reactions were believed to be
uncommonly low, as patients may have avoided admitting
violating medical instructions to avoid sunlight for a
month. Nevertheless, nearly a quarter of the patients
30 reported reactions, most of which occurred within one
month of the treatment. There was "no apparent
relationship of photosensitivity to injected drug dose
... although there may be a trend to less severe
reactions at the lower drug doses." In addition, the
35 length of time to lose photosensitivity may have been
somewhat shorter for the 5 mg/kg group, but it was not

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5 statistically significant. Dougherty concludes that patients should be warned that photosensitivity may last six weeks.

10 Harty et al. treated 7 patients with bladder cancer with an intravenous injection of 2.0 mg/kg of PHOTOFRIN porfimer sodium (one patient received 2/3 of the proper dose), followed 72 hours later by exposure to energy density of 100 J/cm². "Six patients had skin phototoxicity and in each case this occurred within 10 days after administration of [the drug]. Four cases were classified as mild, consisting of erythema and edema of the hands and face and did not require treatment. In 2 patients the phototoxicity was of moderate severity, consisting of second degree burns of hands and face, and required topical therapy." 15 Five patients had irritative bladder symptoms which were associated with loss of smooth muscle and its replacement by fibrous tissue.

20 What is needed is a better method to administer photodynamic therapy to avoid adverse side effects such as normal tissue destruction and photosensitivity reactions. An improved method of therapy also would use a lower light dose, so that treatment could be administered more quickly and efficiently. When the light source emits at a limited power, an improved method would permit shorter light treatment periods and more patients to be treated with the same light source. Another improvement would be a lower dose of the photosensitizing agent, which would lower the cost of treatment and also help avoid side effects.

35 Summary of the Invention

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5 This invention provides a method of
administering photodynamic therapy to an animal. The
method has two steps: First, an effective amount of a
photosensitizing agent is administered to the animal.
The effective amount of the photosensitizing agent in
10 this method is less than about one half of the usual
clinical dose for the same photosensitizing agent.
Second, after a post injection interval of less than
about one fourth the usual interval, an effective dose
of light is administered to the animal. The effective
15 dose of light is less than about one half of the usual
clinical dose of light used in conjunction with the
particular photosensitizing agent.

In another embodiment, the invention is
applied to targets which include, but are not limited
to, tumors, atherosclerotic plaque, localized viral
20 infections, psoriasis, arthritic joints, and ocular
and other neovascularization or hypervascularizations.

While this invention provides for the use of
any photosensitizing agent, preferably the agent is
selected from chlorins (such as chlorin e6),
25 bacteriochlorins, phthalocyanines, porphyrins,
purpurins, merocyanines, pheophorbides, psoralens, and
pro-drugs such as δ -aminolevulinic acid which can
produce drugs such as protoporphyrin in tissue. In
other embodiments, BPD-MA, monoaspartyl chlorin e6,
30 zinc phthalocyanine, tin etiopurpurin, tetrahydroxy
tetraphenylporphyrin, and porfimer sodium are the
photosensitizing agents.

In another embodiment, there is provided a
two-step method for administering photodynamic therapy
35 to an animal. First, a photosensitizing agent is
administered to an animal in an amount sufficient to

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5 produce a photodynamic effect. Second, after a post injection interval of less than about two hours, less than about 75 Joules/cm² of light is administered to the animal.

10 Brief Description of the Drawings

Figure 1 is a representation of a mouse at 48 hours after an injection of 1 mg/kg of BPD-MA and light exposure of 100 Joules/cm² beginning 15 minutes after BPD-MA injection.

15 Figure 2 is a representation of a mouse at 24 hours after an injection of 0.5 mg/kg of BPD-MA and light exposure of 75 Joules/cm² beginning 15 minutes after BPD-MA injection.

Figure 3 is a representation of a mouse at 4 days after an injection of 2.0 mg/kg of BPD-MA and light exposure of 100 Joules/cm² beginning three hours after BPD-MA injection.

20 Figure 4 is a graph showing the BPD-MA dose response curve for doses of 0.5, 1.0, 1.5 and 2.0 m/kg (liposomal formulation). Light exposure took place three hours after injection.

25 Figure 5 is a graph showing the light dose response curve for doses of 50, 75, 100, 125, and 150 J/cm² (at 690nm). Light exposure took place three hours after the injection of 2 mg/kg BPD-MA.

30 Brief Description of the Invention

This invention is a method of administering photodynamic therapy. The method comprises administering a photosensitizing agent and applying radiation to at least a portion of an intact animal at

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5 an intensity effective to impair or destroy
selectively target tissue.

As used herein "target" is that tissue that is intended to be impaired or destroyed by this treatment method. The target takes up the photosensitizing agent; then when sufficient radiation is applied, the target tissue is impaired or destroyed. Targets include, but are not limited to, tumors, atherosclerotic deposits, virus-containing cells such as those infected with papillomavirus (warts), psoriasis, and arthritis. Also included among target cells are rapidly developing capillaries and areas of neovascularization, particularly in the eye. This improved method can be used with the types of tumors with which photodynamic therapy has been used in the past. These tumors generally are rather shallowly located on the body through which the light must penetrate. These include various tumors of the skin, bladder and neck, Kaposi's sarcoma and some esophageal tumors.

25 "Non-target cells" are all the cells of an intact animal which are not intended to be impaired or destroyed by the treatment method. These non-target cells include but are not limited to those of other healthy tissues, including overlying normal skin.

30 "Destroy" is used to mean kill the desired target tissue. "Impair" means to change the target tissue in such a way as to interfere with its function. For example, North et al. observed that after exposure of BPD-treated, virus-infected T cells to light, holes developed in the T cell membrane, which increased in size until the membrane completely decomposed (Blood Cells 18:129-40, 1992). Target

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5 tissues are understood to be impaired or destroyed even if the target cells are ultimately disposed of by macrophages.

"Photosensitizing agent" is a chemical compound which homes to one or more types of selected target tissues and, when contacted by radiation,
10 absorbs the light and induces impairment or destruction of the target tissues. Virtually any chemical compound that homes to a selected target and absorbs light may be used in this invention.

15 Preferably, the chemical compound is nontoxic to the animal to which it is administered or is capable of being formulated in a nontoxic composition.

Preferably, the chemical compound in its photodegraded form is also nontoxic. A comprehensive listing of photosensitive chemicals may be found in Kreimer-
20 Birnbaum, Sem. Hematol. 26:157-73, 1989.

Photosensitive compounds include, but are not limited to, chlorins, bacteriochlorins, phthalocyanines, porphyrins, purpurins, merocyanines, pheophorbides, psoralens and pro-drugs such as δ -aminolevulinic acid,
25 which can produce drugs such as protoporphyrin. A new class of photosensitizing agents, wavelength-specific photosensitive porphycyanine and expanded porphyrin-like compounds is disclosed in U.S. Application docket No. 27301-20078.00, filed 30 October 1992, and
30 incorporated herein in its entirety by reference, can be used in the disclosed method. Preferred photosensitizing agents are benzoporphyrin derivatives (BPD), monoaspartyl chlorin e6, zinc phthalocyanine, tin etiopurpurin, tetrahydroxy tetraphenylporphyrin
35 and porfimer sodium (PHOTOFRIN®). Most preferred is

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5 the benzoporphyrin derivative monoacid ring A (BPD-MA).

"Radiation" or "light" as used herein includes all wavelengths. Preferably, the radiation wavelength is selected to match the wavelength(s) which excite(s) the photosensitive compound. Even
10 more preferably, the radiation wavelength matches the excitation wavelength of the photosensitive compound and has low absorption by the non-target tissues and the rest of the intact animal. For example, the preferred wavelength for BPD-MA is the range of 685-
15 695 nm. A preferred light source is an argon pumped dye laser which is tuned to emit at about 690 nm. Also useful are fluorescent banks of lights, LED panels, and filtered full spectrum arc lamps.

The radiation is further defined in this
20 invention by its intensity, duration, and timing with respect to dosing with the photosensitive agent (post injection interval). The intensity must be sufficient for the radiation to penetrate skin and/or to reach the target tissues to be treated. The duration must
25 be sufficient to photoactivate enough photosensitive agent to act on the target tissues. Both intensity and duration must be limited to avoid overtreating the animal. The post injection interval before light
30 application is important, because in general the sooner light is applied after the photosensitive agent is administered, 1) the lower is the required amount of light and 2) the lower is the effective amount of photosensitive agent.

35 This invention provides a method of treating an animal, which includes, but is not limited to, humans and other mammals. The term "mammals" also

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5 includes farm animals, such as cows, hogs and sheep,
as well as pet or sport animals such as horses, dogs
and cats.

By "intact animal" is meant that the whole,
undivided animal is available to be exposed to light.
10 No part of the animal is removed for light treatment,
in contrast with photophoresis, in which the animal's
blood is circulated outside its body for exposure to
light. The entire animal need not be exposed to
light. Only a portion of the intact animal may or
15 need be exposed to radiation. For discrete tumors and
other conditions affecting a relatively small volume,
it is preferable to apply light solely to the skin
overlying the tumor or other condition.

"Transcutaneously" is used herein as meaning
20 through the skin of an animal.

Typical indications for this treatment
include destruction of tumor tissue in solid tumors,
dissolution of atherosclerotic plaque in blood
vessels, treatment of topical tumors or skin disease
including papillomavirus infections (e.g., warts),
25 psoriasis, arthritis, and conditions characterized by
neovascularization or hypervascularization, particular
of the eyes.

Briefly, the photosensitizing agent is
generally administered to the animal before the animal
30 is subjected to light treatment. Preferably, the
agent is administered at a post injection interval
which is less than one quarter of the usual post
injection interval before subjecting the animal to
light treatment.

35 Preferred photosensitizing agents include,
but are not limited to, chlorins, bacteriochlorins,

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5 phthalocyanines, porphyrins, purpurins, merocyanines,
pheophorbides, psoralens and pro-drugs such as δ -
aminolevulinic acid, which can produce drugs such as
protoporphyrin. More preferred are benzoporphyrin
10 derivatives (BPD) and porfimer sodium. Most preferred
among the benzoporphyrin derivatives is the monoacid
ring A (BPD-MA). Other preferred photosensitizing
agents include but are not limited to monoaspartyl
chlorin e6, zinc phthalocyanine, tin etiopurpurin and
tetrahydroxy tetraphenylporphyrin.

15 The photosensitizing agent is administered
locally or systemically. The photosensitizing agent
is administered gastrointestinally or by injection
which may be intravenous, subcutaneous, intramuscular
or intraperitoneal. The photosensitizing agent also
20 can be administered enterally or topically via patches
or implants. The most preferred method of
administration is intravenous injection.

The photosensitizing agent can be
synthesized as a dimer and thereby absorb more light
on a per mole basis.

25 The photosensitizing agent can be
administered in a dry formulation, such as pills,
capsules, suppositories or patches. The
photosensitizing agent also may be administered in a
liquid formulation, either alone with water, or with
30 pharmaceutically acceptable excipients, such as are
disclosed in Remington's Pharmaceutical Sciences. The
liquid formulation also can be a suspension or an
emulsion. In particular, liposomal or lipophilic
formulations are most preferred. If suspensions or
35 emulsions are utilized, suitable excipients include
water, saline, dextrose, glycerol, and the like.

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5 These compositions may contain minor amounts of nontoxic auxiliary substances such as wetting or emulsifying agents, antioxidants, pH buffering agents, and the like.

10 The dose of photosensitizing agent will vary with the target cell(s) sought, the animal's weight and the timing of the light treatment. For known photosensitizing agents, the effective amount of the photosensitizing agent needed in this method is approximately less than half of the known usual clinical dose. For example, the usual clinical dose is 2.5 mg/kg for porfimer sodium and 0.25 mg/kg for BPD. The effective amount of porfimer sodium in this method is about 0.3 to 1.25 mg/kg. The effective amount of BPD in this method is about .01 to .125 mg/kg. The usual clinical doses for monoaspartyl chlorin e6 (0.1-2.5 mg/kg), zinc phthalocyanine (.5-2 mg/kg), tin etiopurpurin (.5-2 mg/kg) and tetrahydroxy tetraphenylporphyrin (1-5 mg/kg) are halved for use in this method.

25 The dose of light administered also is much lower in this method than in known methods for photodynamic therapy. In general, the light dose is less than about half of the light dose of previous methods. For example, where previously 150 Joules/cm² was used with BPD, the method of the present invention requires no more than 75 Joules/cm². Where previously 10-50 Joules/cm² was used with monoaspartyl chlorin e6, the method of the present invention requires no more than about half of the previous light doses.

35 The duration of radiation exposure is preferably between about 5 and 30 minutes, depending on the power of the radiation source.

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5 The post injection interval in this method
varies by the photosensitizing agent. However, the
post injection interval in one embodiment is less than
about one fourth the clinical post injection interval
used with known photosensitizing agents. For example,
10 the usual clinical post injection interval for BPD is
about 3 hours. In contrast, the post injection
interval for BPD in this invention is less than about
one quarter of that, or less than about 45 minutes.
The usual clinical post injection interval for
15 porfimer sodium is 24-48 hours. In contrast, the post
injection interval in this invention is less than
about 6 hours for porfimer sodium, and preferably less
than about 4 hours.

 This invention is the conduct of effective
20 PDT more safely and with fewer adverse effects because
the post injection interval is much shorter and doses
of both the photosensitive agent and light are halved.
In contrast, previously it was thought that the
photosensitizer initially distributed nonselectively
25 throughout the body and that it took several hours to
days for the photosensitizer to accumulate selectively
in the target tissue. It was thought that selective
distribution occurred gradually, with a considerable
amount of exchange between the target tissue and the
30 pool of circulating photosensitizer molecules. Thus,
it was considered essential to delay post injection
light treatment by several hours to days.

 However, a recent pharmacokinetic study has
brought this long-accepted thinking into question.
35 Richter et al. (Biochem. Pharmacol. (1992) 43:2349-58)
reported that administered BPD has two regioisomers in
equal concentrations. By 3 hours post injection, the

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5 isomer ratio in plasma changes from about 1:1 to
1:0.28, due to liver metabolism. However, when tumor
tissue was removed 15 min and three hours post
injection, and BPD extracted from it, the isomers were
found at essentially equal proportions (1:1.15).

10 While not wishing to be limited by a theory,
the inventors propose these data suggest the
possibility that BPD may accumulate rapidly in tumors,
where it may be immobilized, and permit shorter post
injection intervals.

15 Previously it was assumed that after
injection, photosensitizers first distributed equally
to target and normal tissues. This was the basis for
the assumption that a short post injection interval
would cause extensive damage to normal tissue,
particularly skin.

20 Yet as disclosed in Example 3 of U.S.
Application Serial No. 948,311, mice injected with BPD
can receive relatively high levels of light (about 150
J/cm²) on their shaved backs without any apparent ill
effects so long as exposure takes place within the
25 first two hours post injection (as opposed to the
usual three hours). Blood sampling of those treated
animals indicates that almost 80% of circulating BPD
becomes photobleached by this treatment indicating
that light has activated the drug. Therefore,
30 photosensitizers may not produce generalized tissue
damage even when activated by light, so long as there
is insufficient photosensitizer present in surrounding
cells.

35 These two surprising results encouraged
testing of early, lower dose illumination in tumor
treatment with PDT (i.e., before photosensitizers

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5 permeate skin or other normal tissue). Experimental evidence (presented below) in mice indicates the inventive method is safe and effective.

The examples which follow are intended to demonstrate the efficacy of the invention and to assist in the practice of the invention. The following examples cover one photosensitizing agent and provide a means to screen other photosensitizing agents or new compounds for use in the inventive method. The following examples are intended only to be examples and not to limit the invention in any way.

General Comments

The following general comments on Materials and Procedures apply to Examples 1 and 2, unless otherwise noted.

20 BPD-MA was synthesized as described in U.S. Patents No. 4,920,143 and 4,883,790, incorporated herein by reference. BPD-MA was obtained from QuadraLogic Technologies, Inc. and stored dissolved in DMSO (4.5 mg/ml) at -70°C. Liposomal BPD (4.95 mg/ml) was prepared as described in U.S. Application Serial No. 07/832,542, filed February 5, 1992. The following formula was used:

	<u>Ingredient</u>	<u>Amount (mg/ml)</u>
30	BPD-MA	4.95
	Dimyristoyl Phosphatidyl	23.27
	Choline	
	Egg Phosphatidyl	16.09
	Glycerol	
	Lactose or	148.50
35	Trehalose	
	Ascorbyl Palmitate	0.05

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5 Butylated Hydroxy Toluene 0.005
Water for Injection Q.S.

Liposomal BPD was dried and stored frozen at -20°C in
1 ml aliquots. The appropriate number of aliquots
10 were thawed immediately before use and diluted with 5%
dextrose in water for injection into the animals.

Male DBA/2 mice (7-11 weeks old; Charles
River Laboratories, St. Constant, Quebec, Canada) were
used in these studies, unless otherwise specified.
15 Shaving and depilation removed the hair very
effectively from appropriate body surfaces. The mice
were shaved and depilated with a commercially
available depilator (Nair®) at least one day before
being used in the experiments. Following injection
20 the mice were kept in the dark for various lengths of
time, as described below. Before and after the
experiments the mice were kept in an animal facility
with 12 hours of light and 12 hours of dark daily.

An argon pumped dye laser, whose power
source was obtained from SpectraPhysics (Series 2000,
25 Mountain View, CA) and whose 5W argon ion pumped dye
laser was obtained from Coherent (Model 599, Palo
Alto, CA) was used to deliver a columnated beam of
light having a wavelength of 690 (± 3) nm. The argon
laser was aimed at the skin to irradiate the tumor.
30 The time of light exposure was varied to give
different light doses, such as 50, 75 and 100
Joules/cm².

35 Example 1

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Pilot Study of Shorter Post Injection Intervals

5 DBA/2 mice (weight 22 ± 1 g) were used in this study. First, mice were injected in the flank with M-1 (murine rhabdomyosarcoma) tumor cells and the tumors were allowed to grow to about 5mm in diameter, according to the protocol of Richter et al., Br. J.
10 Cancer (1991) 63:87-93. Mice were injected with liposomal BPD-MA and kept in the dark for 15 minutes before exposure to light. The mice were then treated with the laser.

15 Figure 1 is a representation of a photograph which was taken 48 hours after the mouse was treated with 1 mg/kg of BPD-MA and 100 J/cm² of light. This mouse was alive and shown resting on its belly in profile. Its back still appeared clean-shaven and displayed a large crescent-shaped eschar, which was
20 about the size of its ear. This eschar was located on the mouse's flank where the tumor was eradicated. The tumor was not palpable.

Figure 2 is a representation of a photograph which was taken at 24 hours after treatment with 0.5
25 mg/kg BPD-MA and 75 J/cm² light. This mouse was alive and shown resting on its belly in profile. Its back still appeared clean-shaven and displayed a small, round inflamed, or darkly colored, area located on the mouse's flank where the tumor was eradicated. There
30 was no eschar. The tumor was not palpable. Figure 2 shows that after only 24 hours, the normal skin surrounding the tumor was only slightly inflamed.

Both animals were followed two weeks. The
35 tumors did not grow back; and there was a flat, healing area of skin.

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5 Figure 3 is a representation of a photograph
taken at 4 days after treatment of a mouse with an M-1
tumor. The treatment differed somewhat from the two
preceding regimens. This mouse was treated with 2.0
mg/kg of BPD-MA and irradiated with 100 Joules/cm² of
10 light, administered three hours post injection. This
mouse was alive and shown resting on its belly in
profile. Its back still appeared clean-shaven and
displayed a large crescent-shaped eschar, which was
about the size of its ear. This eschar was located on
the mouse's flank where the tumor was eradicated. The
15 tumor was not palpable.

A comparison of Figure 2 with Figures 1 and
3 graphically demonstrates the reduction in skin
damage when the post injection interval is shortened,
and the doses of BPD-MA and light are reduced.
20

EXAMPLE 2

Dose Ranging Study

Additional DBA/2 mice were prepared with M-1
tumor cells as described above. The tumors were
25 allowed to grow to approximately 5 mm diameter. Then,
the mice were injected with one of two different doses
of BPD-MA (0.5 and 1.0 mg/kg), exposed to one of three
different light doses (50, 75 and 100 J/cm²), and
exposed to light at one of three different post
30 injection intervals (1, 15 and 30 minutes). During
the 15 and 30 minute post injection intervals, the
mice were kept in darkness.

Table 1 shows the number of animals which
were tumor free at each observation period for each
35 drug and light dose and at each post injection
interval. Many of the animals have recently started

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the test. Only a few animals were treated long enough ago to be followed to day 14. Of those, most are tumor free.

Table 1
Interim Test Results for Dose-Ranging
Time-Varying Study

Treatment		Time Post Injection (min.)	Results (# Tumor-Free)		# of Mice
Drug Dose (mg/kg)	Light Dose (J/cm ²)		Day 7	Day 14	
0.5	50	1	3	n/a	3
0.5	50	15	2	n/a	2
0.5	50	30	0	0	2
0.5	75	1	2*	n/a	2
0.5	75	15	2	2	2
0.5	75	30	n/a	n/a	n/a
0.5	100	1	3	n/a	3
0.5	100	15	5	2	5
0.5	100	30	2	n/a	2
1.0	50	1	4	n/a	4
1.0	50	15	4	4	4
1.0	50	30	4	4	5
1.0	75	15	5	4	5
1.0	75	30	4	n/a	5
1.0	100	15	4	4	4

NOTE: (*): observations on Day 2 post exposure
n/a: animals have not been tested or animals have not been in study long enough to reach observation date

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5 At the 0.5 mg/kg BPD-MA dose and 50 J/cm² administered
30 minutes post injection, all mice developed tumors.
Three of five mice given 0.5 mg/kg BPD-MA and 100 J/cm²
administered 15 minutes post injection also developed
tumors by day 14, although all were tumor free at day
10 7.

For comparison, Figures 4 and 5 are
provided. Figure 4 summarizes results of a test
involving the same mouse-tumor model, in which four
different BPD-MA doses were given (0.5, 1.0, 1.5, and
15 2.0 mg/kg). Light exposure was 150 J/cm², administered
after a post injection interval of three hours of
darkness. Under this regimen, which is similar to
current clinical regimens, the only group that was
over 50% tumor free at 14 days was that group of mice
receiving 2.0 mg/kg. This dose was at least double
20 the effective doses displayed in Table 1.

Figure 5 summarizes results of a test
involving the same mouse-tumor model, in which five
different light exposures (50, 75, 100, 125 and 150
J/cm²) were used three hours post injection with 2
25 mg/kg BPD-MA. Under this regimen, which is similar to
current clinical regimens, 75% of the mice receiving
150 J/cm² and 50% of the mice receiving 125 J/cm² were
tumor free at 14 days. These doses of light were
significantly higher than the lowest effective doses
30 displayed in Table 1.

This invention has been described by a
direct description and by examples. As noted above,
the examples are meant to be only examples and not to
limit the invention in any meaningful way.
35 Additionally, one having ordinary skill in this art in
reviewing the specification and claims which follow

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5 would appreciate that there are equivalents to those
claimed aspects of the invention. The inventors
intend to encompass those equivalents within the
reasonable scope of the claimed invention.

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Claims

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1. A method of administering photodynamic therapy to a subject, said method comprising the steps of

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(a) administering to said subject an effective amount of a photosensitizing agent which is less than about one half of the usual clinical dose for said photosensitizing agent; and

15

(b) administering to said subject an effective dose of light which is less than about one half of the usual clinical dose of light used in conjunction with said photosensitizing agent, said light dose commencing at a post injection interval which is less than about one fourth of the usual post injection interval.

20

2. The method of claim 1 wherein said photosensitizing agent is selected from the group consisting of a chlorin, a bacteriochlorin, a phthalocyanine, a porphyrin, a purpurin, a merocyanine, a pheophorbide or a psoralen.

25

30

3. The method of claim 2 wherein said porphyrin is benzoporphyrin derivative mono-acid (BPD-MA) porfimer sodium or tetrahydroxy tetraphenylporphyrin.

35

4. The method of claim 1 wherein said light dose is less than about 75 Joules/cm² or said interval is less than about two hours.

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5 5. A method of performing photodynamic
therapy on a subject, said method comprising the steps
of

 (a) administering to said subject
benzoporphyrin derivative mono-acid in an amount which
is less than about .125 mg/kg; and

10 (b) administering to said subject an
effective dose of light which is less than about one
half of the usual clinical dose of light used in
conjunction with said benzoporphyrin derivative mono-
acid, said light dose commencing after a post
15 injection interval which is less than about one half
hour.

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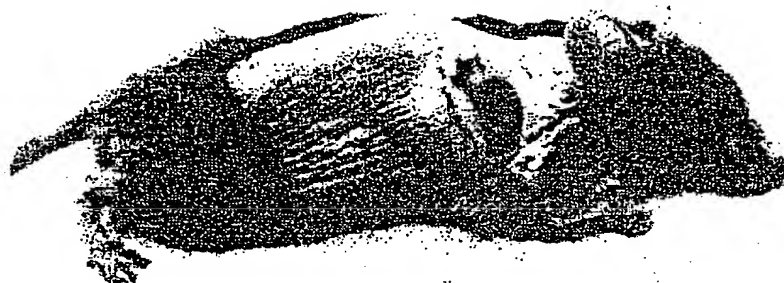


FIG. 1



FIG. 2

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FIG. 3

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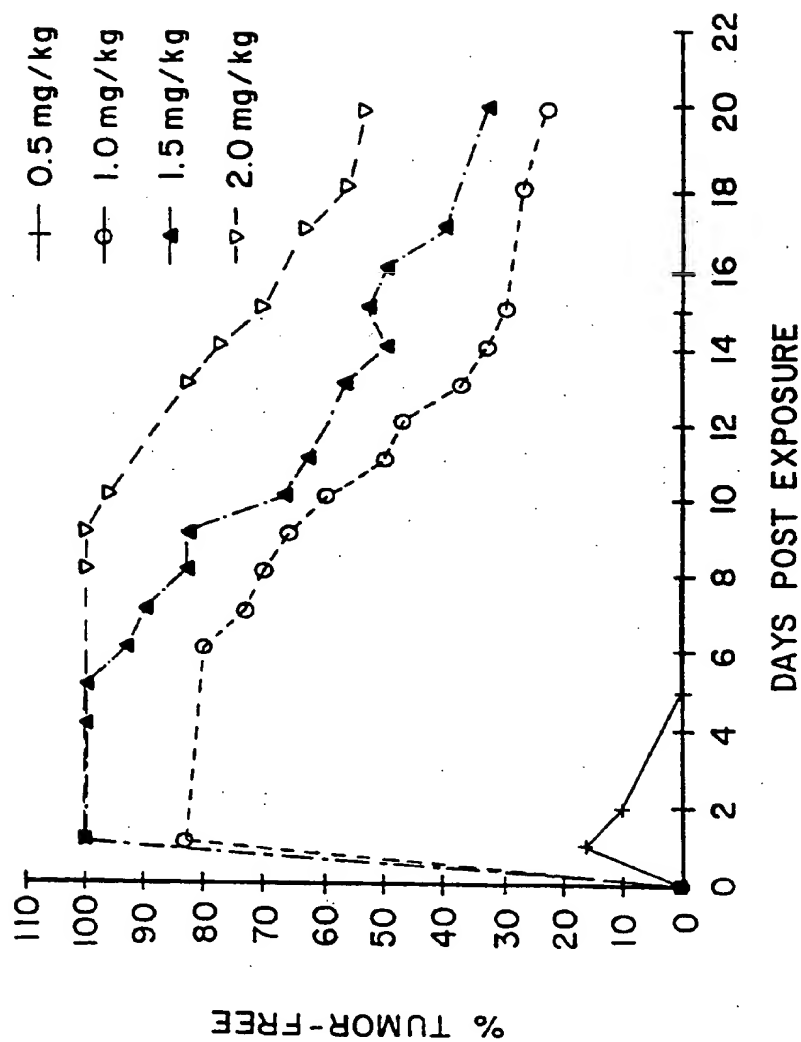


FIG. 4

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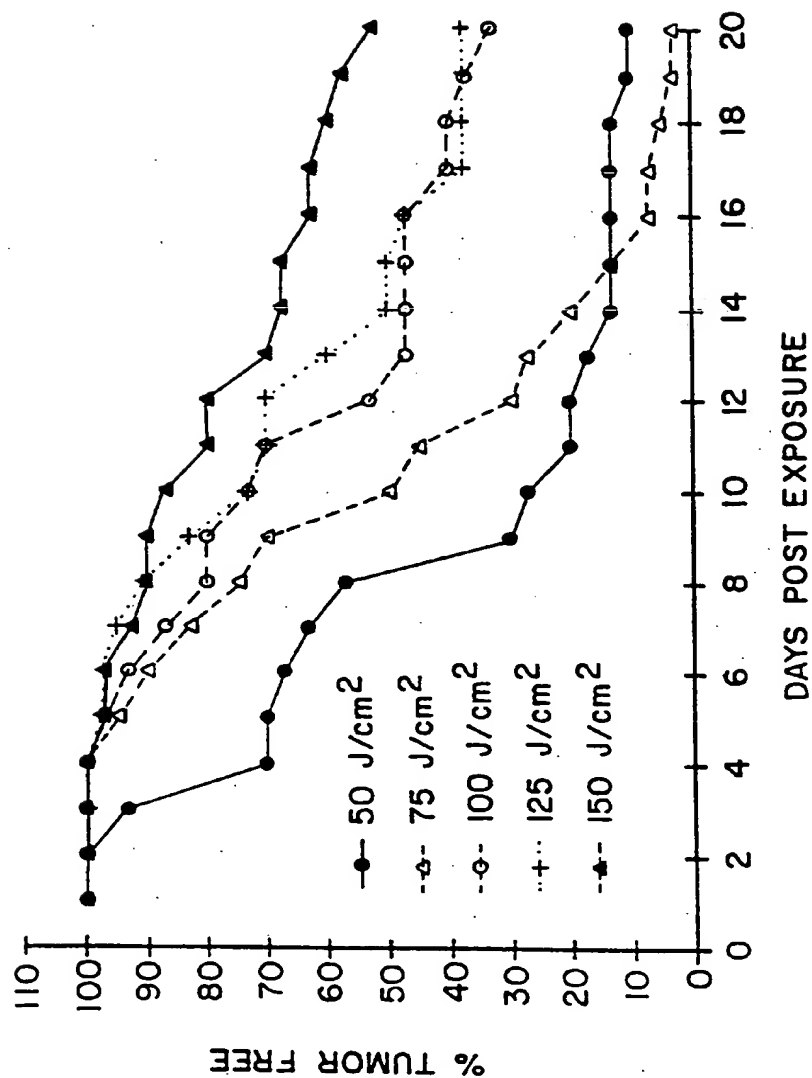


FIG. 5

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INTERNATIONAL SEARCH REPORT

Int. Appl. No.
PCT/CA 93/00490

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 A61N5/06

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 5 A61N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,90 11797 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA) 18 October 1990 see the whole document	1,4
Y	---	2,5
Y	US,A,5 095 030 (J.G. LEVY ET AL.) 10 March 1992 cited in the application see the whole document	2,5
A	---	3
A	EP,A,0 478 506 (TECLAS TECNOLOGIE LASER SA) 1 April 1992 see the whole document	1,2,4,5

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☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
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- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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- *&* document member of the same patent family

Date of the actual completion of the international search

2 March 1994

Date of mailing of the international search report

28. 03. 94

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Ferrigno, A

INTERNATIONAL SEARCH REPORT

Int. Patent Application No.
PCT/CA 93/00490

C(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PROCEEDINGS OF THE IEEE, vol.80, no.6, June 1992, NEW YORK US pages 869 - 889 STUART L. MARCUS 'Photodynamic Therapy of Human Cancer' see the whole document ---	1,2,4,5
A	US,A,4 957 481 (R.A. GATENBY) 18 September 1990 see the whole document ---	1,2
A	EP,A,0 337 601 (EFAMOL HOLDINGS PLC) 18 October 1989 see the whole document ---	2,3
A	US,A,4 973 848 (A.S. KOLOBANOV ET AL.) 27 November 1990 see column 3, line 41 - column 4, line 32 -----	2

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CA93/00490

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 1+5
because they relate to subject matter not required to be searched by this Authority, namely:
See PCT-Rule 39 (IV)
Claims 1 and 5 refer to doses and intervals which are compared with a "usual" one. Any value found in the prior art. would have to be necessarily classified as "usual" this makes meaningless any search for these claims.
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/CA 93/00490

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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EP-A-0337601	18-10-89	AU-A- 3097589 DE-T- 68906301 JP-A- 1275528 US-A- 4992257 US-A- 5162519	14-09-89 30-09-93 06-11-89 12-02-91 10-11-92
US-A-4973848	27-11-90	NONE	

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(21) International Application Number: PCT/CA93/00490 (22) International Filing Date: 17 November 1993 (17.11.93) (30) Priority Data: 07/979,546 20 November 1992 (20.11.92) US (71) Applicant: UNIVERSITY OF BRITISH COLUMBIA [CA/CA]; 2194 Health Science Mall, Vancouver, British Columbia V6T 1Z3 (CA). (72) Inventors: RICHTER, Anna, M.; #903-5775 Toronto Road, Vancouver, British Columbia V6T 1X4 (CA). WATER- FIELD, Elizabeth; 4610 Blenheim Street, Vancouver, British Columbia V6L 3A4 (CA). LEVY, Julia, G.; 2034 West 36th Avenue, Vancouver, British Columbia V6M 1K9 (CA). (74) Agents: ROBINSON, J., Christopher et al.; Fetherstonhaugh & Co., Suite 1010-510 Burrard Street, Vancouver, British Columbia V6C 3A8 (CA).	(81) Designated States: AU, CA, CZ, FI, HU, JP, KP, KR, NO, NZ, PL, SK, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>	
(54) Title: METHOD OF ACTIVATING PHOTOSENSITIVE AGENTS (57) Abstract A method of administering photodynamic therapy begins with administering to an animal an effective amount of a photosensitizing agent which is less than about one half of the usual clinical dose for the photosensitizing agent. Then, following a post injection interval which is less about one quarter of the usual post injection interval, an effective dose of light which is less than about one half of the usual clinical dose of light used in conjunction with the photosensitizing agent is administered to the animal.		